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博 士 学 位 论 文

水体中典型内分泌干扰物的生物有效性评价方法研究

Study on the methods for evaluating the bioavailability of typical endocrine disrupting chemicals in water

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目 录

Contents

摘 要

内分泌干扰物（Endocrine disrupting chemicals,EDCs）由于毒性作用剂量少，潜伏期长，易对人类和生态环境产生灾难性的影响，已经成为 21 世纪各个国家环境科学研究的热点。在我国，EDCs 研究起步比较晚，基础研究比较薄弱，目前还未独立筛选出公认的 EDCs，现有的污染调查及监测数据十分有限，EDCs 的污染现状及其变化趋势还不清楚，形势不容乐观。目前国内外对 EDCs 的研究主要集中在一种或一类 EDCs 的污染状况调查、毒性剂量效应和生态毒理作用上，不能正确反映实际环境中 EDCs 的复合效应。本研究将 EDCs 中不同类型的污染物 PAHs、酚类 EDCs 和重金属作为一个研究整体，分别对其生物有效性评价方法进行了初步研究，为系统研究 EDCs 的复合污染效应提供了方法基础。

本文首先综述了PAHs、酚类EDCs和重金属三种不同类型EDCs的迁移转化和生物有效性评价方法的研究进展；其次，把杂环分子六氢吡啶（Hexahydropiperadine, HHP）和N-乙基哌啶（N-ethylpiperidine, EP）作为第三组分引入β-环糊精（β-Cyclodextrin, β-CD）/α-溴代萘（α-Bromonaphthalene, α-BrN）体系中，建立了新颖的分析卤代多环芳烃（Polycyclic aromatic hydrocarbons, PAHs）的CD-室温磷光法（Room temperature phosphorescence,RTP），探讨了CD-RTP法的发光机理，为评价PAHs的生物有效性提供了方法基础；将半透膜被动采样装置（Semipermeable membrane devices, SPMDs）应用于水体中疏水性较弱的酚类EDCs的检测研究。在实验室静态模拟实验条件下，测定了套筒式SPMD检测酚类EDCs的相关动力学参数，评价了套筒式SPMD模拟生物检测酚类化合物的可行性；在国内首次将激光诱导时间分辨现场荧光监测系统（Laser induced time resolved fluorescence system, LITRFS）应用于九龙江口水体中酚类EDCs的快速检测，比较了LITRFS直接分析水体中酚类EDCs所得结果与传统GC-MS法分析所得结果的相关性，调查了九龙江口水体中烷基酚和BPA（Bisphenol A）的污染状况；将香港城市大学Rudolf研究小组提供的人工贻贝（Artificial mussel, AM）和福建当地的海产品翡翠贻贝（*Perna viridis*）应用于福建泉州湾、九龙江口和东山湾水体中重金属的检测研究，考察了AM模拟生物检测几种典型重金属的可行性，初步调查了上述研究站位水体中重金属的污染状况。主要研究成果归纳如

下：

第一，在β-CD存在下，以荧光量子产率较高的卤代PAHs－α-BrN为模型化合物，以含有孤电子对、杂环的碱性分子HHP或EP为第三组分，在不经除氧条件下考察了温度、包络平衡时间、pH值、第三组分的用量对三元包络物RTP的影响以及不同包络物的磷光寿命差别。实验结果表明，EP、HHP均可作为第三组分诱导α-BrN/β-CD体系产生强的RTP，并应用于α-BrN的分析测定。与传统的CD-RTP法相比，新建立的两种方法具有更高的灵敏度和更低的检测限，对α-BrN分析测定的检测限分别为7.2×10^{-3} μmol/L和1.1×10^{-2} μmol/L，该方法为水环境中PAHs的生物有效性评价方法研究提供了新的方法基础；研究还发现，CD-RTP法还可用于三元包络物中有机小分子EP的直接测定，线性拟合方程为：y=256.8x-12.2（相关系数r= 0.9671），检测限为0.048 μmol/L。将该方法应用于合成样品EP的分析，加标回收率不小于91.4%，样品7次平行测定的相对标准偏差小于2.85%；三元包络物α-BrN/β-CD/EP、α-BrN/β-CD/HHP主要依赖氢键作用力形成，HHP起到封盖保护作用，EP起到封盖保护和空间填充双重作用，α-BrN/β-CD/CH主要依赖疏水性相互作用力形成，CH起到空间填充作用。

第二，将套筒式 SPMD 应用于疏水性较弱的酚类 EDCs，即壬基酚（Nonylphenol，NP）、辛基酚（Ocytlphenol，OP）、双酚 A（Bisphenol, BPA）、2-氯酚（2-Chlorophenol, 2-CP）、2,4-二氯酚（2,4-Dichlorophenol, 2,4-DCP）、2,4,6-三氯酚（2,4,6-Trichlorophenol, 2,4,6-TCP）和五氯酚（Pentachlorophenol, PCP）的检测研究，在实验室静态模拟实验条件下，测定了套筒式 SPMD 检测酚类化合物 NP，OP，BPA，2-CP，2,4-DCP，2,4,6-TCP 和 PCP 的相关动力学参数。结果表明，套筒式 SPMD 用于疏水性较弱的酚类 EDCs 检测具有一定的可行性，酚类 EDCs 的 $\log K_{SPMD}$ 值与自身的 logKow 值呈正相关，相关系数 r 为 0.8494，套筒式 SPMD 在评价水体中酚类 EDCs 生物有效性方面具有一定的应用潜力；把 LITRFS 应用于九龙江口水体中酚类 EDCs 的快速检测，比较了 LITRFS 直接分析水体中酚类 EDCs 所得结果与传统 GC-MS 法分析所得结果的相关性，结果表明 LITRFS 直接分析的水体中酚类化合物的总荧光强度与 GC-MS 法分析得到的总酚浓度具有一定相关性，相关系数 r=0.7311，扣除腐殖质背景干扰后相关系数 r 达 0.8276。以上结果表明，LITRFS 可直接用于调查九龙江口水体中酚类化合

物的污染状况。

第三，将人工贻贝 AM 和福建当地的海产品翡翠贻贝应用于泉州湾、九龙江口和东山湾水体中重金属的检测研究。实验结果表明，翡翠贻贝和 AM 对 Cd，Cu 的检测结果具有很好的相关性，相关系数 r 分别介于 0.8867-0.9984 和 0.9463-0.9871 之间；对 Cr、Zn 的检测结果也具有良好的相关性，相关系数 r 分别介于 0.6828-0.8459 和 0.7716-0.9747 之间；对 Pb 的检测结果相关性波动较大，在九龙江口其相关性很好，r 值达 0.9192，而在泉州湾其相关性不好，东山湾相关性较差，r 值只有 0.5024。以上实验结果证实了 AM 可以模拟生物监测上述三个区域水体中的重金属 Zn、Cu 、Pb、Cr、Cd；由于 AM 克服了生物体自身因素的影响，其分析结果更具规律性；比较国内外不同海域贝类生物体中重金属含量发现，泉州湾、九龙江口和东山湾中重金属 Cu、Zn、Cr 含量与国内外其他海域相当，Cd、Zn、Cu 和 Cr 都没有超标，但 Pb 都超标，而九龙江口 Cd 也已接近预警值。

本文首次将 EDCs 中不同类型的污染物 PAHs、酚类 EDCs 和重金属作为一个研究整体，分别对其生物有效性评价方法进行了初步研究，不仅建立了新颖的可用于评价卤代 PAHs 生物有效性的 CD-RTP 法、实验证实了 SPMD 模拟生物检测水体中酚类 EDCs 基本可行、LITRFS 直接测定水体中酚类 EDCs 和 AM 模拟生物检测水体中重金属的可行性。首次得到了福建泉州湾、东山湾和九龙江口水体中重金属污染状况的第一手资料，为系统研究 EDCs 的复合污染效应提供了新的手段、方法和研究思路。

关键词：内分泌干扰物；多环芳烃；酚类化合物；重金属；人工贻贝；环糊精诱导室温磷光法；激光诱导纳秒时间分辨现场荧光监测系统；生物有效性

Abstract

Endocrine disrupting chemicals (EDCs) could bring drastic effect on species and entironment because of low dosage effect and long delitescence. The study on EDCs has become a hotspot in the 21th century. The study on EDCs has just been payed attention to in China recent years with feeble basic study. We have't screened a kind of EDCs ourselves, have limited pollution investigation and monitoring data, and could't understand the pollution status in China. At present, the study on EDCs mainly has been focused on the pollution of a kind or a sort of EDCs and toxicity effect worldwide. The pollution in real environment is the results of mulriple effects of many different contaminants. The study in the lab could't reflect the pollution effect in real environment accurately. In this study, the methods for evaluating the bioavailability of PAHs, phenolic compounds and HMs were studied respectively with PAHs, phenolic compounds and HMs as a correlative system of EDCs firstly. These methods would provide us with new basis for studying the mulriple effects of different sorts of EDCs in the environment.

In this study, the occurrence, distribution and fate and the methods for evaluating the bioavailability of three different sorts of EDCs, namely, PAHs, phenolic compounds and HMs were summarized at first. Then hexahydropiperadine(HHP) and N-ethylpiperidine(EP) were induced into binary complex β-Cyclodextrin/ α-bromonaphthalene(β-CD/α-BrN) to establish new methods, namely, cyclodextrin induced room temperature phosphorescence(CD-RTP) for monitoring PAHs. At the same time, the luminescence mechanism of CD-RTP was discussed. The semipermeable membrane devices(SPMDs) was used to monitor phenolic compounds-Nonylphenol（NP）, Ocytlphenol（OP）, Bisphenol（BPA）, 2-Chlorophenol（2-CP）, 2,4-Dichlorophenol（2,4-DCP）, 2,4,6-Trichlorophenol（2,4,6-TCP）andPentachlorophenol（PCP）with low hydrophobicity. The kinetic parameters of those phenolic compounds sampled by SPMDs were determined and the feasibility for evaluating the bioavailability of those phenolic compounds sampled by SPMDs was

investigated with static simulating experiments in the lab. Laser induced time resolved fluorescence system（LITRFS）was directly utilized to monitor phenolic compounds in real water environment at Jiulong estuary firstly in China. The correlativity between the data obtained by LITRFS and the data obtained by GC-MS were compared and the pollution status of phenolic compounds at Jiulong estuary was investigated. The Artificial mussel(AM) provided by Rudolf group of City University of Hong Kong and *Perna viridis* were utilized to monitor HMs in Quanzhou Bay, Jiulong estuary and Dongshan Bay. The feasibility for evaluating the bioavailability of HMs by AM and the pollution status of the sampling sites were investigated. Main results were summarized as followings:

(1) α-BrN with high fluorescence quantum yield was selected as PAHs model compound. Strong RTP of α-BrN induced by β-CD in the presence of novel heterocyclic third component, HHP or EP, was firstly obtained respectively without deoxygenization. The effects of equilibrium time for formation of inclusion complex, temperature, pH values, the variation of concentrations of each third component on RTP of ternary inclusion complex and the difference of phosphorescence lifetime of ternary inclusion complex had been investigated and compared. Based on the strong RTP of α-BrN induced by CD in the presence of EP or HHP, two novel CD-RTP methods were established to analyze α-BrN with high sensitivity and low detectability, 7.2×10^{-3}μmol/L and 1.1×10^{-2}μmol/L respectively. These methods provided us new methods references for evaluating the bioavailability of PAHs in water environment. The experimental results also showed that CD-RTP method could be utilized to monitor and identify small organic molecule, EP, in the ternay inclusion complex. The regression equation was $Ip=256.8C_{EP}-12.209$, and the relative coefficient was r=0.9671. With the established CD-RTP method, concentrations of EP in synthetic samples were determined with the recovery not less than 91.4% and the relative standard deviation less than 2.85%(n=7). The formation of ternary inclusion complexes, α-BrN/β-CD/EP and α-BrN/β-CD/HHP, depended on hydrogen bond force with HHP as coverage action and EP as coverage action and spatial-filling

action. The formation of ternary inclusion complexe, α-BrN/β-CD/CH depended on hydrophobic force with CH as spatial-filling action.

(2) The SPMDs was used to monitor phenolic compounds with low hydrophobicity with static simulating experiments in the lab. The obtained kinetic parameters showed that SPMD had big potential to be used to evaluate the bioavailability of phenolic compounds in water. The experimental results also indicated that the $\log K_{SPMD}$ values of phenolic compounds had good correlativity with their own logKow values with the correlative coefficient r=0.8494. LITRFS was directly utilized to quickly monitor phenolic compounds in water at Jiulong esturay. The data obtained by LITRFS has good relativity with the data obtained by GC-MS with the relative coefficient r=0.7311 and r=0.8276 when the background interference of humic acid was eliminated. These results showed that LITRFS could be utilized to monitor phenolic compounds at Jiulong River quickly and conveniently.

(3) The AM and *Perna viridis* were utilized to monitor HMs at Quanzhou Bay, Jiulong estuary and Dongshan Bay. The feasibility for evaluating the bioavailability of HMs by AM and the pollution status of the sampling sites were investigated. The experimental results indicated that Cd and Cu monitored by AM had very good relativity with that monitored by *Perna viridis* and the relative coefficient r was between 0.8867 and 0.9984, 0.9463 and 0.9871 respectively. Cr and Zn monitored by AM had good relativity with that monitored by *Perna viridis* and the relative coefficient r was between 0.6828 and 0.8459, 0.7716 and 0.9747 respectively. Pb monitored by AM had very good relativity with that monitored by *Perna viridis* at Jiulong River with the relative coefficient r=0.9192, and the relativity at Quanzhou Bay was just ordinary but was very poor at Dongshan Bay with the relative coefficient r=0.5024. These experimental results validated AM could be used to evaluate the bioavailability of Cd, Cu, Cr, Zn and Pb in water. AM could overcome the interference of biology factors, so the analytical results by AM for HMs had better orderliness than that by *Perna viridis*. Compared to the pollution level reported in other research, the pollution level of Cu, Cr, Zn at three study sites was equivalent to

that reported worldwide. The concentrations of Cd, Cu, Cr, Zn were not beyond the set standard but Pb was beyond the set standard at the three study sites. The concentration of Cd was oncoming to the set standard at Jiulong River.

In this study, the methods for evaluating the bioavailability of PAHs, phenolic compounds and HMs were studied respectively with PAHs, phenolic compounds and HMs as a correlative system of EDCs firstly. We have established new methods, CD-RTP, for evaluating the bioavailability of PAHs, made sure that SPMD had big potential to be used to evaluate the bioavailability of phenolic compounds in water, LITRFS could be utilized to monitor phenolic compounds quickly and conveniently, and AM could be used to evaluate the bioavailability of HMs in water. At the same time we got the data of the pollution status of HMs at Quanzhou Bay, Dongshan Bay and Jiulong River, in Fujian firstly. All these work will provide us with the novel means, methods and research idea for studying the mulriple effects of different sorts of environmental contaminants.

Key words: Endocrine disrupting chemicals; Polycyclic aromatic hydrocarbons; Phenolic compounds; Heavy metals; Artificial mussel; Cyclodextrin induced room temperature phosphorescence; Laser induced time resolved fluorescence system; Bioavailability

第一章 绪 论

1.1 内分泌干扰物(Endocrine disrupting chemicals，EDCs)概述

由于人类的生产和生活活动而释放到周围环境中，对维持生物体内平衡、生殖、发育及行为的正常激素的合成、分泌、结合、清除等功能产生影响，从而影响内分泌系统的化学物质，通称为内分泌干扰物，又叫环境激素、环境荷尔蒙或内分泌紊乱物质等[1,2]。内分泌干扰物按其生物活性可分为类雌激素及抗雌激素、类雄激素及抗雄激素、类甲状腺素及抗甲状腺素等，目前主要研究的大都是环境雌激素。早在上世纪30年代，二羟联苯的雌激素效应就已有报道[3]。而后，70年代发现了乙烯雌酚综合症，80年代在英国的沙布塔河发现了鲤鱼雌雄同体的现象，90年代在佛罗里达洲和五大湖发现爬行类和鱼类的雌性化现象。此外，著名的“水俣病”和海豹肢畸形婴儿的大量出现、妇女乳腺癌发生率增加、男性精子数量和质量下降等现象也被认为与环境激素的污染有关。因此环境激素被人们称为“威胁人类存亡的定时炸弹”，EDCs已经成为迫切需要治理的第三代环境污染物[4]。上世纪90年代以来，环境化学物质的内分泌干扰效应引起了学术界和公众的极大关注，也引起了发达国家政府、WHO等机构的高度重视。关于化学污染物是否通过与内分泌系统交互作用，对人类和野生生物产生不良健康效应的争论，已经得到国际上生态学家、内分泌学家、毒理学家、流行病学家的极大关注，并在世界范围内展开了广泛深入的调查研究，成为国际研究的热点[5]。英、美、日等国家先后都召开了有关EDCs的科学研讨会，为了建立试验准则和筛选具有内分泌干扰作用的各类农药及化学品，美国环保局还组建了一个有关内分泌干扰物的专家特别委员会。1995年，美国政府设立了由环保局领导14个政府部门组成的工作组来研究控制EDCs的污染问题，并于1996年建立食品中的EDCs的筛选方法，日本也于1997、1998年相继发表、公布了有关环境EDCs的报告书。而1996年美国科学家Colborn等[6]发表的《Our Stolen Future》一书，极大程度地促进了美、欧、日等发达国家对EDCs污染问题的重视和研究，被认为是EDCs研究中的里程碑。很多国际组织和政府机构就EDCs的暴露、对人类和野生动物内